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Heterotopic Cardiac Transplantation

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An abbreviated version of this protocol was published in Frontiers in Immunology in Jan 2019

Relative Frequencies of Alloantigen-Specific Helper CD4 T Cells and B Cells Determine Mode of Antibody-Mediated Allograft Rejection

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Detailed protocol

Background

Abdominal heterotopic heart transplantation in rats was described by Abbott in 1964 (1), and then in mice by Corry in 1973 (2). Even though more technically demanding, the murine model is more widely used to study allograft vasculopathy (AV) due to the large number of transgenic and gene knockout strains, e.g. mice lacking IFN-γ showed the importance of this cytokine in AV (3). The technique involves placing the donor heart in the abdomen, where the donor ascending aorta is anastomosed end-to-side to the abdominal aorta, and the pulmonary artery is anastomosed end-to-side to the recipient inferior vena cava. Blood enters the donor ascending aorta from the recipient abdominal aorta and is diverted into the coronary arteries by the closed aortic valve. After the myocardium is perfused, venous blood drains into the right atrium through the coronary sinus and is pumped back into the recipient inferior vena cava by the right ventricle. As the aortic valve is initially competent and blood does not enter the left ventricle, the allograft lacks 'volume loading,' and thus does not represent the physiology in human orthotopic transplantation (4).

Methodology

Transplants were carried out using an operating microscope (Carl Zeiss OPMI1-FC, Thornwood, NY, USA) at a magnification of between x6 to x40. Details of the procedure are described below.

Donor operation

Anaesthesia was induced and maintained with isofluorane delivered through a nose cone. Sterile conditions were maintained throughout the operation. The abdomen was opened with a midline incision and the abdominal vena cava dissected from the retroperitoneum. Unfractionated heparin (200 units, LEO Pharma A/S, Ballerup, Denmark) was injected into the vena cava followed by compression for thirty seconds to allow systemic circulation of the injected heparin. The abdominal cava and aorta were then divided and the donor exsanguinated. Inferior vena cava (IVC), left superior vena cava and right superior vena cava were ligated and divided in order, using 7/0 vicryl suture (Ethicon, Edinburgh, UK). The ascending aorta was transected proximal to the origin of the right brachiocephalic artery and the main pulmonary artery was transected proximal to the division into right and left pulmonary arteries. Branches of the pulmonary veins were ligated *en masse* with a single 7/0 vicryl suture, tied to the base of the heart. The heart was removed and placed in sterile saline at 4°C until implantation.

Recipient operation

Anaesthesia was induced and maintained with inhalational isofluorane. Recipients were given buprenorphine analgesia (Temgesic, Schering-Plough, Harefield, UK) [3 mL of 1mg/mL buprenorphine was added to 7 mLs of water injection, and administered subcutaneously at a dose of 0.01 mL per 10g body weight). Animals were placed in a supine position on a heated (37°C) operating board, and sterile conditions were maintained throughout. The abdomen was opened with a midline incision, and after blunt dissection of the retroperitoneum, the infra-renal abdominal aorta and IVC were identified. Two microsurgical vascular clips were applied approximately 1 cm apart across both the aorta and IVC, and an aortotomy performed. The donor heart was removed from cold saline and the donor aorta sutured to the recipient aorta in an end-to-side fashion, using continuous 10/0 nylon BearTM sutures (Bear Medic Corp, Tokyo, Japan). A venotomy was performed and an end-to-side anastomosis of the donor pulmonary artery to recipient IVC performed, using continuous 10/0 nylon sutures. After the establishment of donor heart sinus rhythm, the abdominal wall and skin were closed in two layers, using 5/0 vicryl sutures. The recipient animal was administered subcutaneously normal saline (0.5 ml) for fluid replacement. The animal was then placed in an incubator heated to 27°C until recovery was complete.

The duration of cold ischaemia (from placement of the heart in cold saline until removal and commencement of the anastomosis) was typically 20-25 minutes. The duration of warm ischaemia (time taken to complete the anastomosis) was approximately 60 minutes. Transplant function was evaluated by daily abdominal palpation. Mice with non-functioning grafts within two days of transplantation were considered technical failures and were excluded. Rejection was defined as complete cessation of palpable myocardial contraction and confirmed by laparotomy at the time of explant.

References

1. Abbott CP, Lindsey ES, Creech O, Jr., & Dewitt CW (1964) A Technique for Heart Transplantation in the Rat. *Arch Surg* 89:645-652.
2. Corry RJ, Winn HJ, & Russell PS (1973) Primarily vascularized allografts of hearts in mice. The role of H-2D, H-2K, and non-H-2 antigens in rejection.*Transplantation* 16(4):343-350.
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4. Asfour B*, et al.* (1999) A simple new model of physiologically working heterotopic rat heart transplantation provides hemodynamic performance equivalent to that of an orthotopic heart. *J Heart Lung Transplant* 18(10):927- 936.

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1. Motallebzadeh, R. and Pettigrew, G. (2021). Heterotopic Cardiac Transplantation. Bio-protocol Preprint. bio-protocol.org/prep1170.
2. Alsughayyir, J., Chhabra, M., Qureshi, M. S., Mallik, M., Ali, J. M., Gamper, I., Moseley, E. L., Peacock, S., Kosmoliaptsis, V., Goddard, M. J., Linterman, M. A., Motallebzadeh, R. and Pettigrew, G. J.(2019). Relative Frequencies of Alloantigen-Specific Helper CD4 T Cells and B Cells Determine Mode of Antibody-Mediated Allograft Rejection. Frontiers in Immunology 9. DOI: 10.3389/fimmu.2018.03039